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IMP production and energy metabolism during exercise in rats in relation to age

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IMP production in and force exerted by rat quadriceps muscle *in situ* during various types of exercise were examined in relation to age. During continuous isometric exercise with constant stimulation time, the amount of IMP was linearly and inversely related to the age of the animals; a higher IMP concentration was found in intermittent isometric and dynamic exercise. No relationship was found between the total AMP deaminase activity and age. Exercise influenced neither the total activity nor the activity in the soluble fraction. From the results it is concluded that: (1) the IMP concentration is linearly related to the free intracellular ATP^{4-}/ADP^{3-} ratio and the free AMP^{2-} concentration; (2) older animals are better able to maintain a high intramuscular ATP^{4-}/ADP^{3-} ratio and a low AMP^{2-} concentration; (3) IMP is produced in particular under conditions when the muscle has to work under extreme stress. IMP possibly exerts a feed-back control on the contraction system.

INTRODUCTION

Since the work of Lowenstein (Goodman & Lowenstein, 1977; Lowenstein, 1972) it has been recognized that the decrease in total adenine nucleotide concentration during exercise is due to deamination of AMP to IMP, catalysed by AMP deaminase (AMP aminohydrolase, EC 3.5.4.6):

$$AMP + H_2O \rightarrow IMP + NH_3$$

This reaction, which is irreversible, is one of the reactions of the purine nucleotide cycle (Goodman & Lowenstein, 1977; Lowenstein, 1972). Re-amination of IMP takes place via reactions catalysed by adenylosuccinate synthase (EC 6.3.4.4) and adenylosuccinase (EC 4.3.2.2) respectively. Furthermore, IMP can be degraded to hypoxanthine and urate, which are subsequently excreted.

The activities of adenylosuccinate synthase and adenylosuccinase are relatively low compared with the AMP deaminase activity (Goodman & Lowenstein, 1977; Lowenstein, 1972), which is not evenly distributed in muscles. The highest activity, found in fast-twitch fibres (Meyer & Terjung, 1979; Meyer *et al.*, 1980), is proposed to be functional in keeping the relative adenine nucleotide concentration optimal for contraction (Goodman & Lowenstein, 1977; Lowenstein, 1972). Fast-twitch fibres contract fast and therefore show a high rate of ADP production. Via the adenylate kinase reaction, ADP can be converted back into ATP and AMP ($2\ ADP \rightleftharpoons ATP + AMP$). Thus the contribution of adenylate kinase and AMP deamination prevents an increase in ADP and AMP, both inhibitors of myosin ATPase.

In the course of a series of experiments on the relationship between mechanical output and energy utilization with young rats (47 ± 2 days old) (H. G. Westra, A. de Haan, J. E. van Doorn & E. J. de Haan, unpublished work) and on the metabolic effects of training with old rats (79 ± 4 days old) (Westra *et al.*, 1985), the IMP concentration after continuous isometric contractions in the old rats appeared to be less than in the young rats. In the present paper experiments are reported in which this observation was elaborated by measuring the IMP production during continuous and intermittent isometric and dynamic contractions of the quadriceps muscle *in situ* of rats ranging in age between 21 and 94 days old.

Since one of the reasons for a decrease in IMP production with increasing age might be a lower AMP deaminase activity in older animals, this activity was measured after contraction and in the resting muscle of animals of different age.

MATERIALS AND METHODS

Surgical preparation

Male Wistar rats fed *ad libitum* and 21–94 days old (45–420 g) were used. The age of the animals was calculated from the growth curve based on measurements of 681 animals. Between body weight (BW, in g) and age (A, in days) there is a linear relationship: $BW = 5.03\ A - 57.95$ ($r = 0.99$), with a S.D. of about 9%.

The rats were anaesthetized with sodium pentobarbital (60 mg/kg body wt., intraperitoneally). The skin of both upper limbs was removed. In the exercise studies the left quadriceps muscle (mixed fast-twitch) was prepared free at the patellar end without damaging the tissue and connected to a force transducer to measure the force developed during contraction.

The stiffness of the transducer system was 726.37 $N \cdot cm^{-1}$, resulting in a shortening of the muscle by less than 1% of its initial muscle length.

The rats were secured by fixing the hip bone with pins to the operation platform. The origin of the quadriceps muscle to the hip bone and femur was left intact.

Exercise studies

Two platinum electrodes were twisted around either end of the muscle. The contractions were induced by square wave pulses from a stimulator (Farnell, Wetherby, W. Yorkshire, U.K.) with a pulse duration of 0.5 ms, a pulse frequency of 80 Hz and a pulse intensity of 1 mA. Three series of experiments were carried out under anaerobic conditions (the blood supply to the muscle was interrupted): continuous isometric exercise (Series A), intermittent isometric exercise (Series B) and dynamic exercise (Series C). In Series A the stimulation time was 14 ± 0.5 s; in Series B contractions were induced by 30 0.5 s contractions with 10 s rest in between. During the experiment the force was registered continuously. Dynamic exercise (Series C) was induced by 15 or 30 1 s contractions with 1 s rest in between. Force, shortening velocity and shortening distances were not measured.

In Series A and B, twitches were used to estimate the optimum muscle length (L_0).

Freeze-clamping

Metabolism was arrested by rapid freeze-clamping of the contracting muscle tissue, by using a pair of clamps pre-cooled in liquid N_2. The exact fixation time was recorded as a result of the disturbance of the force transducer during freeze-clamping. The right quadriceps muscle was used for resting control values. To avoid influences of stimulation, the control muscle was freeze-clamped just before the left leg was stimulated.

Analytical methods

Metabolites. The muscle samples were freeze-dried (VirTis apparatus) and stored at -18 °C until assay. ATP, ADP, AMP, phosphocreatine, creatine and lactate in neutralized extracts were assayed by the methods described by Bergmeyer (1970). Control experiments indicate that on addition of creatine to extracts of freeze-clamped muscle the recovery was about 95% and that phosphocreatine was stable in the acid extracts under our conditions.

IMP in the muscle extracts was determined after separation from the other nucleotides by t.l.c. on polyethyleneimine–cellulose sheets (20 cm × 20 cm; Bakerflex, Phillipsburg, NJ, U.S.A.). After pre-elution of the plates with distilled water, 25 μl samples were applied, together with a standard of IMP. After elution with 0.5 M-sodium formate buffer (pH 3.4), IMP and other nucleotides were localized under u.v. light (254 nm) and the IMP spots were marked. The marked spots were scanned with a Shimadzu dual-wavelength scanner at 260 nm (reference wavelength 360 nm). This method gave 97% recovery with respect to standard IMP solutions analysed enzymically.

AMP deaminase activity. This was assayed by measuring the amount of NH_3 produced during incubation of a tissue extract in the presence of AMP. The freeze-clamped muscle was powdered in a mortar while liquid N_2 was continuously added. The powder was homogenized with a Potter–Elvehjem homogenizer and a Teflon pestle in a buffer (50%, v/w) containing 100 mM-triethanolamine, 5 mM-EDTA, 10 mM-$MgCl_2$, 150 mM-KCl, 0.1% bovine serum albumin, pH 6.5. We used 150 mM-KCl as an approximation to the intracellular conditions. In the same medium the homogenate was sonicated for 3 × 10 s, with 50 s rest in between.

Table 1. Concentrations of metabolites of the quadriceps muscle *in situ* at rest

Values are means ± S.D. ($n = 16$).

Metabolites	Concn. (μmol/g dry wt.)
Phosphocreatine	80.2 ± 7.1
Creatine	65.1 ± 5.4
Phosphocreatine + creatine	145.3 ± 10.0
Creatine/phosphocreatine	0.82 ± 0.08
Lactate	7.9 ± 2.8
ATP	31.1 ± 2.5
ADP	4.9 ± 0.3
AMP	0.4 ± 0.1
ATP + ADP + AMP	36.4 ± 2.6
ATP/ADP	6.32 ± 0.43
IMP	< 0.1
ATP + ADP + AMP + IMP	36.4 ± 2.6

For the total activity measurements 500 μl of the homogenate was added to 4.5 ml of the same buffer. In several experiments the homogenate was centrifuged (10 min, 18000 g, -4 °C) and the activity in the supernatant was measured; 1 ml of supernatant was added to 4 ml of the same buffer. The sediment was homogenized in 4.5 ml of the same buffer and sonicated as described above. Measurement of the activity in the sediment + supernatant showed a recovery of 99 ± 3%.

The activity measurement was carried out at 37 °C. After 3 min of preincubation, the reaction was started by adding 500 μl of 50 mM-AMP (dissolved in the same buffer). After 10 min the reaction was stopped by adding 10% (w/v) $HClO_4$/10 mM-EDTA and neutralized with 5 M-K_2CO_3/5 M-KOH. The time between freeze-clamping of the muscle and the start of the incubation was about 30 min. In the neutralized extracts NH_3 was measured as described by Bergmeyer (1970). The AMP deaminase activity was expressed as nmol of NH_3 produced/min per g wet wt.

Materials

Enzymes and nucleotides were supplied by Boehringer, Mannheim, Federal Republic of Germany, and other chemicals by BDH Chemicals, Poole, Dorset, U.K.

Calculations and statistical analysis

The results are given as means ± S.D. Where appropriate, the Δ metabolite concentrations were calculated by taking the difference between individual values during contraction and the mean value at rest. Where appropriate, statistical comparison between groups were made by Student's t test. A Pearson's correlation coefficient and the 95% confidence interval were used for these comparisons. Linear regression analysis was carried out by the least-squares method.

RESULTS

Metabolite concentrations at rest and after exercise

Values for the contents of phosphocreatine, creatine, ATP, ADP, IMP and lactate of the muscle at rest are given in Table 1. No dependence on age could be detected, therefore the mean values are given. The values are comparable with values reported in the literature for man (Harris *et al.*, 1974; Karlsson *et al.*, 1975) and for

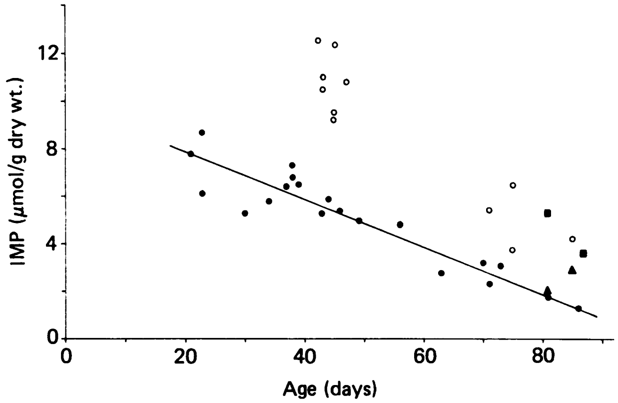

Fig. 1. IMP concentration in the electrically stimulated quadriceps muscle *in situ* in the rat after a 15 s exercise, in relation to age

The exercise consisted of continuous isometric contractions (Series A: ●), intermittent isometric contractions (Series B: ○) and dynamic contractions (Series C: ▲, 15 s; ■, 30 s). For Series A: IMP = $(-0.10 \times \text{age}) + 9.76$ ($r = -0.93$).

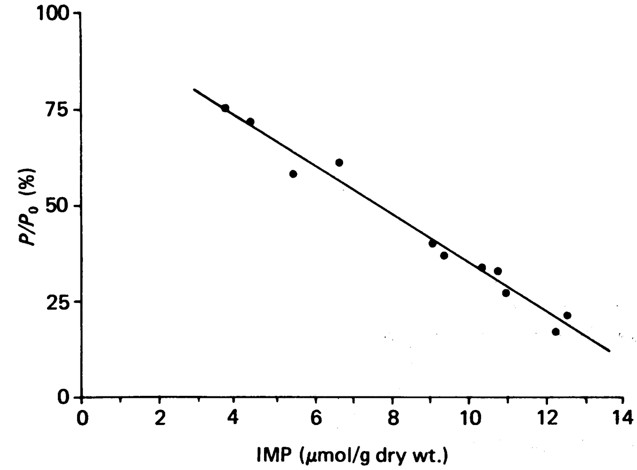

Fig. 2. The ratio of maximal force of the last exercise bout to the maximal force of the first exercise bout (P/P_0, %) of the electrically stimulated quadriceps muscle *in situ* of the rat during intermittent isometric exercise in relation to the IMP concentration

For Series B: $P/P_0 = -6.4 \times [\text{IMP}] + 98.6$ ($r = 0.99$).

rats (Goodman & Lowenstein, 1977; Hohorst *et al.*, 1962).

In agreement with data in the literature (Lowenstein, 1972; Goodman & Lowenstein, 1977; Meyer & Terjung, 1979; Meyer *et al.*, 1980; Westra *et al.*,, 1982, 1985), we found that the amounts of phosphocreatine and ATP decreased and those of creatine, ADP, AMP, IMP and lactate increased during exercise forced by electrical stimulation. However, the amount of IMP formed during exercise appeared to be dependent on the age of the animal. Fig. 1 shows the IMP concentration after continuous isometric exercise in relation to age and the effect of intermittent isometric and dynamic exercise, and that after a 15 s continuous isometric exercise the IMP concentration decreased with increasing age. The higher IMP concentration coincided with a lower ATP concentration, whereas the sum of the concentrations of ATP, ADP, AMP and IMP was independent of the age of the animals.

Except for lactate, no relationship with age could be detected for the changes in the other metabolites after contraction. The 15 s continuous isometric exercise showed that up to the age of 40 days the lactate concentration increased, the value after 40 days stabilizing around 50–60 μmol/g dry wt. In some experiments the muscles were forced to carry out intermittent isometric (Series B) or dynamic (Series C) exercise. Analysis showed that under those conditions the decrease in the phosphocreatine content of the muscle was about 15% greater than during continuous isometric exercise. The lactate concentration in Series B and C was about twice that in Series A, with a higher increase in the younger animals. Fig. 1 shows that also the IMP concentration under those conditions was doubled, in young as well as in old animals.

During the experiments of Series B it appeared that there were large differences in the extent to which the muscles were able to sustain the force. This can be expressed as the ratio of the maximal force during the last exercise bout to the maximal force during the first contraction (P/P_0). It appeared that this ratio was highly correlated with the formation of IMP during exercise. This is shown in Fig. 2, suggesting that IMP is formed in particular under conditions when the muscle tends to be exhausted.

AMP deaminase activity

One reason for a lower IMP concentration in older animals might be a lower AMP deaminase activity. However, measurements of the total activity (0.49 ± 0.07 mmol of NH_3/min per g wet wt.; $n = 23$) in the crude homogenate of the muscle at rest showed no dependence on age.

Since it was reported that exercise influenced the ratio of bound/free AMP deaminase activity (Rahim *et al.*, 1979; Shiraki *et al.*, 1979), and furthermore that association of the enzyme with myosin increased the activity (Shiraki *et al.*, 1979), we also determined the activity after contraction. This activity (0.45 ± 0.06 mmol of NH_3/min per g wt wt.; $n = 10$) was not significantly different from the value at rest, and was independent of age. The activity was also measured in the soluble fraction obtained after centrifugation of the muscle homogenate for 10 min at 18000 g. The activity in the soluble fraction was the same (0.22 ± 0.04 mmol of NH_3/min per g wet wt.) irrespective of whether the fractions were obtained from muscles at rest ($n = 23$) or after exercise ($n = 10$).

Relationship between IMP and adenine nucleotides

The variation in IMP formation is caused by the influence of the metabolite concentrations on the AMP deaminase activity, since the maximal activity of the enzyme in muscles of rats of different ages is the same (results not shown). Since AMP deaminase activity in the intact muscle not only is a function of the AMP concentration, but is also inhibited by ATP and stimulated by ADP (Coffee & Solano, 1977; Wheeler & Lowenstein, 1980), we decided to calculate the free concentrations of these adenine nucleotides in the rat muscles of different ages under various conditions.

The free ATP^{4-}, ADP^{3-} and AMP^{2-} concentrations were calculated as described by McGilvery & Murray (1974) and Goodman & Lowenstein (1977), assuming that the creatine kinase and myokinase reactions were in equilibrium. In our calculations we have taken the equilibrium constants as given by Goodman & Lowenstein (1977). Furthermore, an intracellular Mg^{2+} concentration of 0.5 mM, an extracellular space of 13% (Westra *et al.*, 1978) and a wet-weight/dry-weight ratio of 4.17 is assumed (Westra *et al.*, 1982). Since the adenine nucleotide concentration in the mitochondrial fraction in the muscle is about 5% of the total adenine nucleotide concentration, we have taken the total adenine nucleotide concentration for our calculations. As the free adenine nucleotide concentrations are pH-dependent, the pH is calculated from the lactate concentration (ignoring the contribution of pyruvate) by using the equation given by Sahlin *et al.* (1975):

$$pH = -0.00532 \times [\text{lactate} + \text{pyruvate}] + 7.06$$

with [lactate+pyruvate] concentrations in μmol/g dry wt. The free ADP^{3-} and AMP^{2-} concentrations that we calculated by this procedure are about 2% and 1.2% of the total ADP and AMP concentrations respectively. These percentages are in close agreement with those calculated by Goodman & Lowenstein (1977), although our absolute values are somewhat higher.

In Figs. 3 and 4 the relationships are presented between the amount of IMP in the muscle and the calculated free ATP^{4-}/ADP^{3-} ratio and the free AMP^{2-} concentration respectively. In Figs. 3 and 4 experiments are included that were performed with rat muscles of different ages, the stimulation time was varied, muscles were stimulated continuously as well as intermittently, and furthermore the muscles performed static or dynamic exercise. Figs. 3 and 4 show that a linear relationship was found between IMP and the ATP/ADP ratio and the AMP concentration, irrespective of the conditions applied.

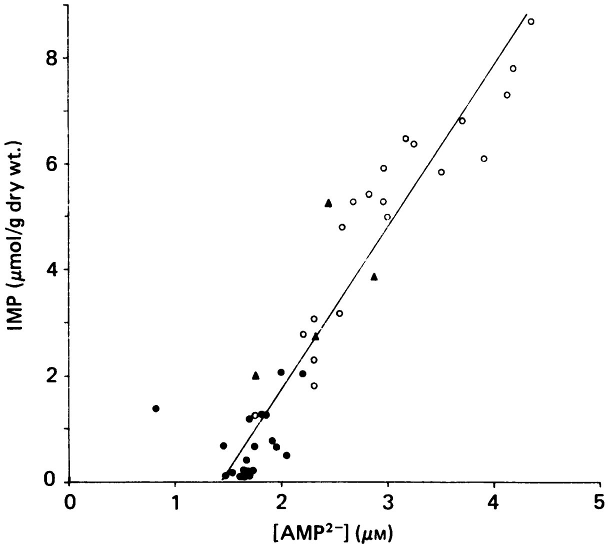

Fig. 4. Free AMP^{2-} concentration in the electrically stimulated quadriceps muscle *in situ* of the rat in relation to the IMP concentration

In previous experiments with old rats (79±4 days, ●; Westra *et al.*, 1985), the stimulation time was varied between 0.9 and 30 s. In the present study in Series A (age 21–86 days, ○) the stimulation time was about 15 s. The exercise consisted of continuous isometric exercise. In Series C (age 84±3 days; ▲) the exercise consisted of dynamic exericise. For all experiments $n = 43$.

$$[IMP] = 3.05 \times [AMP^{2-}] - 4.33 \; (r = 0.95).$$

We have omitted point (0.86; 1.4) from our calculations.

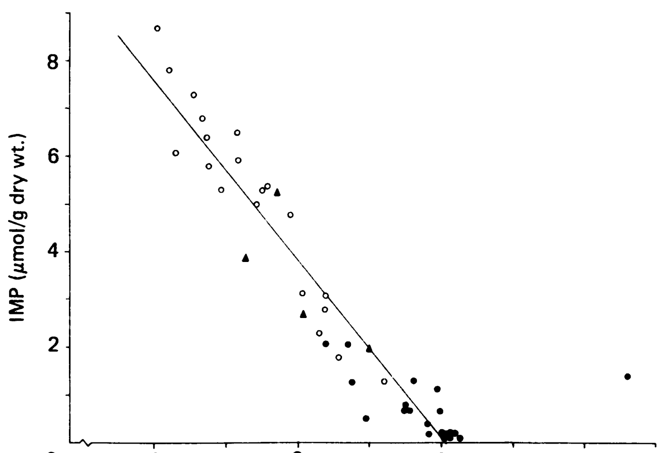

Fig. 3. Free $[ATP^{4-}]/[ADP^{3-}]$ ratio in the electrically stimulated quadriceps muscle *in situ* of the rat in relation to the IMP concentration

In previous experiments with old rats (79±4 days, ●; Westra *et al.*, 1985), the stimulation time was varied between 0.9 and 30 s. In the present study in Series A (age 21–86 days, ○) the stimulation time was about 15 s. The exercise consisted of continuous isometric exercise. In Series C (age 84±3 days, ▲) the exercise consisted of dynamic exercise. For all experiments $n = 43$.

$$[IMP] = -1.87 \times ([ATP^{4-}]/[ADP^{3-}]) + 15.05 \; (r = -0.96).$$

We have omitted point (10.6; 1.4) from our calculations.

DISCUSSION

The IMP production during exercise was examined. The exercise consisted of maximal tetanic continuous isometric contractions, intermittent isometric contractions or dynamic contractions of the fast-twitch quadriceps muscle of the rat. Because of the difference in age of the animals, the amount of stimulated muscle mass was different. The question arose whether the muscles carried out the same relative amount of exercise. There are three arguments which show that this is the case. First, measurements show that the maximal force per 100 g body wt. in both continuous and intermittent exercise (7.87±0.75 N; $n = 31$) was independent of age. Experiments with the gastrocnemius medialis muscle of the rat (A. de Haan, R. Rexwinkel, J. E. van Doorn, A. J. Sargeant, A. P. Hollander, P. A. Huying, R. D. Woittiez & H. G. Westra, unpublished work) revealed a linear relationship between muscle weight and body weight. Second, during continuous exercise the relative force at the end of the 15 s contraction, expressed as percentage of the maximal force (P/P_0, %) was independent of age (23±3%, $n = 20$). Third, metabolic changes during continuous exercise showed that the total energy utilization, calculated from the changes in the concentrations of phosphocreatine, ATP, IMP and lactate, was independent of age, except for the very young animals ($n = 3$), where it was about 15% lower.

Comparison of the IMP concentrations at different ages shows that the IMP production decreases linearly with increasing age of the animals (Series A). The lower IMP concentration coincides with a higher ATP concentration, the sum of the concentrations of ATP+ADP+AMP+IMP being independent of age. Thus breakdown of IMP to hypoxanthine and urate is negligible. From the work of Lowenstein's group (Goodman & Lowenstein, 1977; Lowenstein, 1972) it is also known that re-synthesis of adenine nucleotides from IMP is very low compared with IMP formation. We therefore conclude that the lower IMP concentration in older animals is due to a lower rate of IMP production.

Measurement of the AMP deaminase activity in muscle homogenates revealed that the total activity before and after contraction is independent of age. Also the ratio bound/free enzyme activity was the same whether it was measured in resting muscle or after stimulation. This finding is in contrast with the results of Shiraki *et al.* (1981) and Rahim *et al.* (1979). An explanation for the different results might be that Shiraki *et al.* (1981) regarded the activity in the supernatant after centrifugation of the muscle homogenate in a high-salt medium (300 mM-KCl) as the total activity. However, in our hands this procedure solubilized only $58 \pm 5\%$ ($n = 12$) of the total activity of the homogenate. Thus a considerable fraction of the enzyme was still bound to the particulate fraction. The 60% increase in the total activity after contraction found by Rahim *et al.* (1979) cannot be explained by the results presented here.

Our results suggest that the large differences in IMP production are not correlated with the differences in the amount of AMP deaminase activity as measured under saturating conditions. However, in the cell the AMP deaminase is likely to be influenced by the relative concentrations of the adenine nucleotides (Coffee & Solano, 1977; Wheeler & Lowenstein, 1980). Indeed, the IMP formation in the muscles shows a linear relationship with the calculated intracellular free ATP^{4-}/ADP^{3-} ratio as well as with the free AMP^{2-} concentration (Figs. 3 and 4). The IMP formation may be directly influenced by the intracellular AMP^{2-} concentration, the latter being the substrate of the AMP deaminase. Indeed, the intracellular AMP concentration ranges in our experiment from 1.5 to 4.5 μM, whereas with the isolated enzyme a K_m value of about 0.4–0.8 mM was estimated (Coffee & Solano, 1977; Wheeler & Lowenstein, 1980).

On the other hand, the studies with isolated AMP deaminase have shown that ATP and ADP are potent regulators of the enzyme, even at low concentrations (K_i of ATP about 2–5 μM; half-maximal stimulation by about 0.1 mM-ADP) (Coffee & Solano, 1977; Wheeler & Lowenstein, 1980). Extrapolation of these kinetic data to the intact cell is hampered by the fact that the enzyme activity is influenced by other cell substituents, such as K^+, GTP, P_i and phosphocreatine. It is therefore also possible that the linear relationship found in Fig. 3 expresses the regulatory properties of ATP and ADP on AMP deaminase. Our experiments do not allow discrimination between both possibilities. The results of Figs. 3 and 4 show that the high IMP production in young animals, as shown in Fig. 2, is accompanied by a low ATP/ADP ratio and a high AMP concentration. As discussed above, muscles from young and old animals perform the same relative amount of exercise. Apparently, young animals are not sufficiently able to keep the energy state of cell high enough in the first seconds of exercise.

Fig. 2 shows a significant correlation between the IMP concentration at the end of the intermittent isometric exercise and the P/P_0 ratio. Assuming that the decrease in the P/P_0 ratio may be taken as a measure of the work-load laid on the muscle in relation to its capacity, the correlation suggests that IMP is produced in particular under conditions when the muscle has to work under extreme stress. This idea is supported by the observation that intermittent exercise causes a greater production of IMP (Fig. 1) and lactate and a higher utilization of phosphocreatine than does continuous exercise of the same contraction time.

We therefore suggest that IMP exerts a feed-back control, signalling to the contraction system that work is being done under exhausting conditions.

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